Efficacy and safety of rituximab in type II mixed cryoglobulinemia

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The best treatment of type II mixed cryoglobulinemia (MC) has still to be defined. Antiviral treatment for the frequent underlying infectious trigger hepatitis C virus (HCV) may be ineffective, contraindicated, or not tolerated in a fraction of cases, whereas current immunosuppressive treatments may lead to relevant complications. Selective B-cell blockade with rituximab was used in this study, based on favorable results in preliminary experience. Fifteen consecutive patients with type II MC (HCV-related in 12 of 15) were treated with rituximab, 375 mg/m^2 intravenously weekly for 4 weeks. Only medium- to low-dose steroids were allowed, if already administered at the time of recruitment. All patients had active disease, poorly controlled or difficult to manage with previous treatments, including corticosteroids in all. Efficacy and safety of rituximab therapy were evaluated in the following 6 months. The overall follow-up after rituximab treatment ranged from 9 to 31 months. Rituximab proved effective on skin vasculitis manifestations (ulcers, purpura, or urticaria), subjective symptoms of peripheral neuropathy, low-grade B-cell lymphoma, arthralgias, and fever. Nephritis of recent onset went into remission in one case. Laboratory features, that is, significantly decreased serum rheumatoid factor and cryoglobulins and increased C4, were consistent with the clinical efficacy. Treatment was well tolerated, with no infectious complications. Thrombosis of retinal artery or self-limiting panniculitis occurred in one patient each. Rituximab may represent a safe and effective alternative to standard immunosuppression in type II MC. Controlled studies are needed to better define drug indications and the cost-efficacy profile in the different systemic manifestations. (Blood. 2003;101:3827-3834)

Introduction

Type II mixed cryoglobulinemia (MC) is a systemic vasculitis prevalently mediated by immune complexes, usually associated with hepatitis C virus (HCV) infection, and characterized by nonneoplastic proliferation of rheumatoid factor (RF)–positive B-cell clones leading to cryoglobulin production.[1,2]

The treatment of HCV-associated MC with severe organ involvement remains difficult at present and may target either the viral trigger HCV, when present, or the downstream pathogenic events by means of less specific approaches such as corticosteroids, immunosuppressors, or plasmapheresis.[1]

There is clinical evidence that effective anti-HCV treatment with interferon (old studies)[1,3-6] or interferon plus ribavirin (recent studies)[6-8] often gave positive results on MC, but results may differ for the different organ manifestations and, rarely, some manifestations are worsened or even directly induced by interferon.[9,10] Antiviral therapy also may prove ineffective, contraindicated, or not tolerated. Furthermore, it does not allow a rapid improvement in rapidly progressive or life-threatening MC manifestations, and it does not represent an option in the small subset of HCV-unrelated type II MC. On the other hand, current therapies with corticosteroids, cyclophosphamide, and plasmapheresis may lead to life-threatening complications (including major infections and cytopenias), may enhance viral replication, and are difficult to manage in the long term.[1,4,6,11-13] Finally, such therapies may prove ineffective or poorly/not tolerated as well. Thus, more effective and less toxic approaches are needed.

One of them might be represented by rituximab, a human-mouse chimeric monoclonal antibody that reacts with CD20 antigen, a transmembrane protein present in different maturation steps of B lymphocytes (from early pre-B to mature lymphocyte), thus directly and selectively targeting the B cells.[14-16] Rituximab proved effective and very well-tolerated in B-cell non-Hodgkin lymphomas, and it also has been successfully used in some autoantibody-mediated autoimmune diseases.[15-17] With particular regard to autoimmune diseases characterized by pathogenic rheumatoid factors, that is, rheumatoid arthritis and type II MC, preliminary results have been encouraging, and clinical efficacy was accompanied by biologic evidence of effective targeting of the RF-positive B-cell population.[18-20] In the present study, we report clinical and biologic data on rituximab treatment in a larger cohort of patients with type II MC. Based on these results, rituximab deserves additional investigation as a safe and effective alternative to standard immunosuppressive therapy for this condition.

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Table 1. Main clinical and laboratory data on the studied patients

Patient	Age/sex	Disease duration (mo)	HCV genotype	Previous therapies	Main clinical features at baseline	Serum laboratory features at baseline				Follow-up after RTX (mo)
						Cryo mg/dL	RF IU/mL	IgM g/L	C4 mg/dL	
1	58/M	49	1b	IFN/Cy/CS/PF	Purpura, fever, arthralgias, nephritis	90	868	1.7	8	15
2	67/F	240	ND	CS/PF	Purpura, urticaria	<50	42	1.0	2	31
3	68/F	20	HCV neg	CS/PF/IVIG	Purpura, skin ulcers, neuropathy	1500	79	14.0	2	30
4	68/F	72	ND	CS	NHL, immune anemia, and neutropenia	446	101	8.0	6	21
5	70/F	132	2c	CS/AZA	Purpura, NHL, neuropathy, arteriopathy	595	552	4.5	2	16
6	63/F	36	1b	CS/IFN/IVIG/PF	Purpura, neuropathy	2280	206	4.3	2	12
7	43/F	168	1b	IFN/CS/Cy	Purpura, skin ulcers	1305	197	1.6	2	12
8	62/M	144	1b	CS/CVP/PF/IFN-R	NHL, hyperviscosity, astenia	+(NA)	216 000	84.0	NA	19
9	66/F	108	HCV neg	AZA/Cy/CS/CSA/IFN	Purpura, neuropathy, arthralgias	258	414	1.2	2	9
10	79/M	84	2a2c	CS	Purpura, skin ulcers, neuropathy	770	1920	1.1	6	9
11	63/F	144	1b	IFN/PF/CS/IVIG	Neuropathy, nephritis	2993	3700	4.4	12	9
12	73/F	144	ND	PF/IFN/CS/ 2-CdA	Purpura, skin ulcers	60	26	1.7	3	9
13	69/F	96	3a	IFN/CS/PF/IVIG	Purpura, skin ulcers, neuropathy	1858	3080	3.8	8	9
14	64/M	8	HCV neg	CS	Purpura, neuropathy, arthralgias	156	526	2.2	2	9
15	53/F	192	2c	IFN/CS	Purpura, arthralgias	2605	545	1.3	7	9

RF indicates rheumatoid factor (positive if ≥ 20 IU/mL); C4, complement C4 fraction (decreased if < 10 mg/dL); RTX, rituximab; IFN, interferon alpha; Cy, cyclophosphamide; CS, corticosteroids; PF, plasmapheresis; ND, not determined; IVIG, intravenous immunoglobulins; NHL, non-Hodgkin lymphoma; AZA, azathioprine; CVP, cyclophosphamide, vincristine, prednisone; R, ribavirin; NA, not available due to serum gelification; CSA, cyclosporin-A; and 2CdA, 2-Chlorodeoxyadenosyne.

Patients and methods

Patients

Fifteen consecutive unselected patients with type II MC unresponsive to conventional treatments were treated with rituximab (Table 1). Preliminary results in 4 of them have been previously reported.[20] Patients were 11 women and 4 men, all human immunodeficiency virus (HIV) negative, with a mean age of 64.4 years (range, 43-69 years) and a mean disease duration of 8.5 years (range, 8 months-20 years) (Table 1). Type II MC was associated with HCV chronic infection in 12 cases, with positive anti-HCV antibodies by enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ) and by recombinant-based immunoblot assay (Ortho Diagnostic Systems), and with positive serum HCV RNA by nested polymerase chain reaction (PCR), according to published procedures (Table 1).[21] HCV genotyping was done as previously described,[21] and serum HCV RNA was quantified before and after treatment (at baseline and at months +3 and +6) by quantitative PCR (Amplicor HCV test; Roche Diagnostics, Basel, Switzerland). In 1 of the 3 remaining patients type II MC was associated with Sjögren syndrome, whereas the remaining 2 cases had a truly "essential" form.[1] One patient (patient 4) had positive anti–hepatitis B virus antibodies, whereas no patient proved positive for the hepatitis B virus antigens (HBsAg, HBeAg) in the serum.

Clinical, laboratory, and instrumental evaluation for what concerns the clinical features of type II MC[2,21] extensively characterized all the patients.

The end point of the study was the evaluation of rituximab clinical efficacy and safety in such type II MC patients nonresponsive to standard treatments. The duration of the study was 6 months. Whenever possible, the duration of disease response, if any, was evaluated during a long-term follow-up (ie, more than 6 months) in the lack of any additional treatment.

Treatment with rituximab and follow-up

Approval was obtained from the Policlinico Universitario-University of Udine institutional review board for these studies. Informed consent to treatment was obtained from all patients according to the Declaration of Helsinki. The therapeutic schedule consisted of the administration of rituximab (Mabthera; Roche, Milan, Italy) 375 mg/m^2 intravenously at days +1, +8, +15, +22, as in treatment of B-cell lymphoma.[15,16] Only medium- to low-dose corticosteroids (prednisone, < 0.5 mg/kg/d) were allowed as concomitant treatment for type II MC if already administered, with no further increase in dosage. Patients were evaluated at baseline and then monthly for 6 months. Premature treatment withdrawal was allowed at any point, based on the decision of either the physician or the patient when side effects occurred, and always in the presence of any toxicity of grade IV by the World Health Organization (WHO) or a neurotoxicity grade of 3 or higher.

A complete physical and laboratory examination was performed at baseline and then monthly.

With regard to the cutaneous manifestations of type II MC, the number and the diameter of skin ulcers were recorded, and purpura was scored as +++ (diffuse and persistent involvement of the trunk and the lower limbs), ++ (diffuse and persistent involvement of the lower limbs), + (limited or fluctuating involvement of the lower limbs), and 0 (no purpura).

Lymphoma features were searched at baseline by means of physical examination, laboratory tests, bone marrow biopsy, and by chest and abdomen computed tomography in all patients.[21] Lymphoma was assessed by tissue biopsy and pathologic evaluation, and computed tomography was repeated at the end of month +6 for restaging.

Neuropathic symptoms (pain and paresthesias) were graded according to a patient-scored visual analog scale (VAS) (range, 0-10). A response was considered present if a decrease greater than 25% in VAS was noticed. Muscle strength was evaluated according to the Medical Research Council of Great Britain in 6 grades, from 0 (complete paralysis) to 5 (normal strength).[22] Furthermore, electromyography of upper and lower limbs was performed at baseline and, whenever possible, was repeated at month +6 after rituximab treatment.[23]

Articular pain also was graded according to a patient-scored VAS, as in the case of neuropathy symptoms.

The evaluation of type II MC–associated glomerulonephritis included both laboratory tests and renal biopsy.

A routine laboratory evaluation, including serum liver enzymes and albumin, was performed every week in the first month, and then monthly. Additional serologic tests included rheumatoid factor by nephelometry (positive if ≥ 20 IU/mL), characterization and quantification of serum cryoglobulins, C3 and C4, protein electrophoresis, and immunoglobulin levels (IgG-IgA-IgM) according to published methods.[2,19,21]

Bone marrow biopsy was performed in all the patients at baseline. Flow cytometry analysis of lymphoid markers (CD3, CD4, CD8, CD19, CD20, CD5, CD23, kappa and lambda) in bone marrow mononuclear cells was performed both at baseline and at the end of month +6 in 7 cases. The same lymphoid markers were evaluated in peripheral blood mononuclear cells at

baseline, and then monthly, in all the patients during the same 6-month period.

Statistical analysis

A paired Student *t* test was used to assess the significance of change in laboratory tests after treatment.

Results

Clinical manifestations

All but one patient completed the full course of rituximab therapy. Although patient 1 underwent only 2 weekly infusions of rituximab,[18] the patient's data was included for evaluation.

Follow-up after treatment ranged from 9 months to 31 months (Table 1).

A rapid response in cutaneous vasculitis was observed in all patients.

Purpura disappeared within months +1 and +2 in 11 of 12 cases (Table 2). Skin ulcers healed in 5 of 5 patients, with improvement beginning within month +2 in all, and complete healing of all the ulcers was obtained within month +3 or +4 (Table 2). Purpura recurred in 2 of 11 responders during month +5 or +6, whereas skin ulcers recurred in 1 of 5 responders at month +6 (Table 2). Urticarial vasculitis rapidly improved and disappeared in patient 2 by the end of month +1 and did not recur.

Two complete responses and one partial response were observed in the 3 patients with low-grade B-cell lymphoma associated with type II MC (Table 1). In patient 4, a complete response of parotid lymphoma was obtained within month +2, but lymphoma recurred in laterocervical lymph nodes at month +6, and radiotherapy was then used. Hemolytic anemia also was present in this patient at baseline, and complete response was noticed from month +2. In patient 5, peripheral monoclonal B-cell lymphocytosis disappeared by month +2, and no recurrence was noticed at the end

Table 2. The course of the main clinical manifestations in the studied patients after rituximab therapy

Patient	Clinical status	Baseline	+90 days	+180 days
1	Purpura	+++	+	+++
	Nephritis: creatinine-24 h proteinuria-urine sediment	2.2 mg/dL–2.6 g-active	2.2 mg/dL–3.6 g-active	2.5 mg/dL–3.4 g-active
	VAS (0-10) for arthralgias	9	2	9
	Fever	> 38°C	0	0
2	Purpura	++	0	0
	Vasculitic urticaria	+ widespread	0	0
3	Purpura	+++	0	++
	Skin ulcers (number)	5	2	0
	VAS (0-10) for neuropathic pain/paresthesias	8/5	3/2	3/2*
4	B-cell NHL, extranodal marginal zone	Stage IV	CR	relapse
	Immune anemia	Hb 104 g/L	Hb 126 g/L	Hb 133 g/L
	Immune neutropenia	Neutrophils 0.2 × 10^9/L	Neutrophils 1.3 × 10^9/L	Neutrophils 1.4 × 10^9/L
5	Purpura	+	0	0
	B-cell NHL, immunocytoma	Stage IV	CR	CR
	VAS (0-10) for neuropathic pain/paresthesias	6/6	6/6	2/0*
6	Purpura	+	0	0
	VAS (0-10) for neuropathic pain/paresthesias	5/7	4/4	4/4*
7	Purpura	+++	0	0
	Skin ulcers (number)	6	3	0
8	B-cell NHL, immunocytoma	Stage IV	PR	PR
	Serum hyperviscosity (normal values, 1.4-1.8)	13.8	ND	3.8
9	Purpura	+	0	0
	VAS (0-10) for neuropathic pain/paresthesias	9/9	3/3	7/7
	VAS (0-10) for arthralgias	8	2	4
10	Purpura	++	0	0
	Skin ulcers (number)	2	1	0
	VAS (0-10) for leg pain (neuropathy + ulcers)	6	3	0
11	VAS (0-10) for neuropathic pain/paresthesias	9/9	1/0	0/0
	Nephritis: creatinine-24 h proteinuria-urine sediment	0.8 mg/dL–1.5 g-active	0.9 mg/dL–0.18 g-inactive	0.8 mg/dL–0.05 g-inactive
12	Purpura	++	0	0
	Skin ulcers (number)	5	0	1
13	Purpura	++	0	+++
	Skin ulcers (number)	1	0	0
	VAS (0-10) for neuropathic pain/paresthesias	4/8	1/5	2/5
14	Purpura	+	0	0
	VAS (0-10) for neuropathic pain/paresthesias	ND/6	ND/4	ND/3*
	VAS (0-10) for arthralgias	8	0	0
	Fever	> 38°C	0	0
15	Purpura	+	0	0
	VAS (0-10) for arthralgias	6	0	0

Purpura was scored as +++, ++, + as reported in "Patients and methods."

VAS indicates patient-scored (range, 0-10) visual analog scale; 0, absent; CR, complete response; PR, partial response; NHL, non-Hodgkin lymphoma; and ND, not determined.

*Electromyography was repeated at month +6 in these patients (pattern unchanged with respect to baseline).

Table 3. Corticosteroid treatment before and after rituximab therapy in the studied patients

	Corticosteroid therapy previously used to treat the MC manifestations present at baseline			Corticosteroid use after RTX (dose in PDN mg/d)*						
Patient	Duration	CS monotherapy	Effect	0	+1	+2	+3	+4	+5	+6
1	PDN 25 mg/d in the previous 12 mos	Yes	Ineffective on any manifestation; PDN tapered and stopped before RTX	0	0	0	0	25	25	25
2	PDN up to 25 mg/d for 8 y	Yes	Mild improvement in purpura and urticaria; PDN tapered and stopped before RTX	0	0	0	0	0	0	0
3	PDN up to 75 mg/d for 5 mos	No (CS + IVIG)	Partial efficacy on purpura; onset and worsening of ulcers and neuropathy	25	0	0	0	0	0	0
4	PDN up to 75 mg/d for 3 mos	Yes	Efficacy on immune anemia and neutropenia, but relapse after PDN suspension	0	0	0	0	0	0	0
5	PDN 12.5 mg/d for 11 y	No (CS + AZA)	Partial efficacy on purpura; worsening of neuropathy	5	5	0	0	0	0	0
6	PDN up to 20 mg/d for 3 y	No (CS + PF; CS + IVIG)	Partial efficacy on purpura; worsening of neuropathy	17.5	2.5	0	0	0	0	0
7	PDN up to 25 mg/d for 12 y	No (CS + IFN; CS + Cy)	Ineffective	12.5	0	0	0	0	0	0
8	CVP chemotherapy 4 y before	No (CVP)	Partial decrease of hyperviscosity, then relapse	0	0	0	0	0	0	0
9	PDN up to 10 mg/d for 8 y	Yes	Partial efficacy on purpura and arthralgias; neuropathy worsened	5	0	0	0	0	0	0
10	PDN up to 25 mg/d for short periods	Yes	Partial efficacy on purpura; onset of neuropathy and skin ulcers	0	0	0	0	0	0	0
11	PDN up to 25 mg/d for 9 y	Yes	Sensory neuropathy worsened; then PDN tapered and stopped 12 mos before RTX, with onset of motor neuropathy and nephritis	0	0	0	0	0	0	0
12	PDN up to 25 mg/d for 8 y	Yes	Partial efficacy on purpura; onset of skin ulcers; PDN tapered and stopped before RTX	0	0	0	0	0	0	0
13	PDN up to 25 mg/d for 6 y	Yes	Partial efficacy on purpura; onset of skin ulcers; worsening of neuropathy	2.5	1.25	0	0	0	0	0
14	PDN up to 15 mg/d for 7 mos	Yes	Ineffective	12.5	0	0	0	0	0	0
15	PDN up to 12.5 mg/d for 15 y	Yes	Effective, but relapse for PDN doses <12.5 mg/d	10	5	7.5	2.5	2.5	7.5	5

RTX indicates rituximab; PDN, prednisone; CS, corticosteroids; IVIG, intravenous immunoglobulins; AZA, azathioprine; PF, plasmapheresis; IFN, interferon alpha; Cy, cyclophosphamide; and CVP, cyclophosphamide, vincristine, prednisone.

*0, +1 to +6 refer to corticosteroid use at baseline and at the end of months +1 to +6 after the beginning of rituximab treatment.

of month +6. By contrast, lymphoma response in patient 8 was delayed: serum M component decreased between month +5 and month +6, with concomitant dramatic decrease in serum viscosity (Table 2). He had active liver disease, cytopenia, a previous myocardial infarction, and aortic aneurysm. Angiography followed by noninvasive treatment of the aortic aneurysm, by intravascular prothesis, could be successfully performed.

A response in symptoms of peripheral neuropathy was recorded in 7 of 7 patients. Changes in neuropathy symptoms were difficult to score in one additional patient (patient 10) (Table 2). A decrease of 50% or more in neuropathic pain was recorded in 6 of 7 patients, with complete disappearance in one of them at month +6 (Table 2). Similarly, a decrease in paresthesias at the lower limbs was noticed in 7 of 7 cases, with complete disappearance in 2 of 7 at month +6 (Table 2). Improvement usually was noticed between months + 2 and +3. Improvement was still present at the end of month +6 in all patients. All patients had normal muscle strength (grade 5) by physical examination at baseline, except for patient 11, whose motor neuropathy led to clinical complaints of severe fatigue, inability to walk up the stairs, and inability to walk on her toes and heels. A marked decrease in fatigue and a marked improvement in muscle strength in the lower limbs were recorded from month +2, with recovery in the above-mentioned disturbances in deambulation. Electromyography confirmed the diagnosis of chronic polyneuropathy in 8 of 8 tested patients at baseline, was consistent with axonal plus myelinic damage, and detected a sensory and a mild motor involvement in all (more pronounced in patient 11). The electromyography pattern was unchanged with respect to baseline in 4 of 4 patients who agreed to repeat the test at month +6 (Table 2).

Among the 2 patients with glomerulonephritis (membranoproliferative pattern), a rapid response with disappearance of proteinuria and an inactive urinary sediment was observed in patient 11, where active nephritis was of recent onset (1 month). By contrast, no improvement was noticed in patient 1, where rituximab was interrupted after the first 2 infusions due to side effects, and where nephritis was of long duration and with renal insufficiency at baseline.[18]

Improvement or disappearance of arthralgias was recorded in 4 of 4 patients shortly after the beginning of rituximab treatment, that is, within month +1, but recurrence was then observed in 2 cases (Table 2). Fever disappeared within month +1 in 2 of 2 patients and did not recur (Table 2).

Corticosteroid sparing effects of rituximab

All patients had been treated with corticosteroids before rituximab, and corticosteroids had proved ineffective or difficult to manage in the long term in all. Corticosteroid treatment was maintained at baseline in 8 patients and could then be reduced and suspended in 7 of them shortly after rituximab treatment (Table 3). In addition, corticosteroids were no longer required in 3 of 4 patients where steroids had been tapered and suspended immediately before rituximab therapy. Finally, 3 patients were off steroids for more than one year before rituximab treatment and remained steroid free (Table 3).

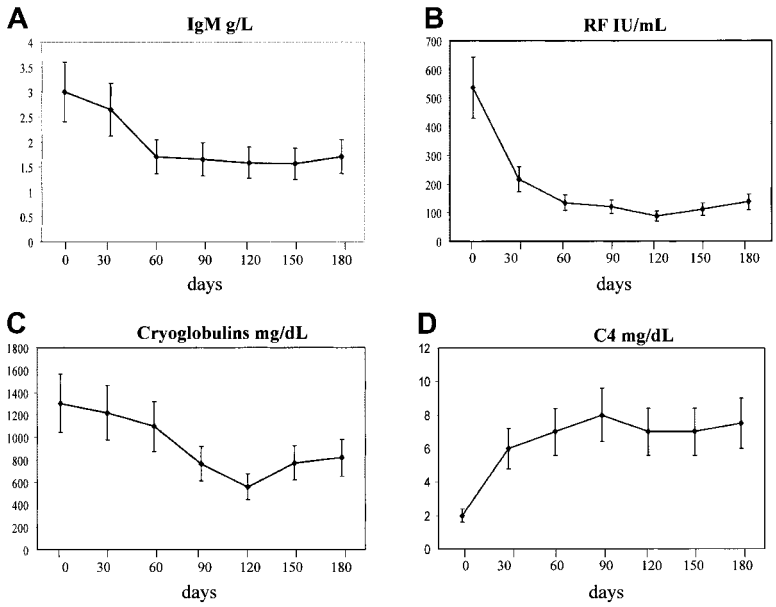

Figure 1. Median values (with standard error bars) at baseline and during the 6-month follow-up in the studied patients. (A) Serum IgM. (B) Rheumatoid factor (RF). (C) Cryoglobulins. (D) C4.

Laboratory features

B-cell depletion in the peripheral blood B cells was achieved in all the patients from the first control at the end of month +1 and was still present at the end of month +6 in all (not studied at month +6 in patient 1; median value of $CD20^+$ cells: 0.2×10^9/L at baseline; 0.008×10^9/L at month +1; 0.001×10^9/L at month +6; $P < .005$ at any monthly control).

By contrast, bone marrow $CD20^+$ cells were depleted in 2 of 7 cases at month +6 (19% and 6% at baseline; 0% and 1% at month +6 in patients 5 and 7, respectively). If compared to baseline values, however, the percentage of $CD20^+$ cells in the bone marrow was decreased (> 25%) at month +6 in 6 of 7 cases (median value at baseline, 12%; range, 6%-38%; median value at month +6, 6%; range, 0%-12%; a value of 7% was noticed both at baseline and at month +6 in patient 11).

A significant reduction in the serum levels of RF, cryoglobulins, and IgM was noticed after treatment, in conjunction with a significant increase in the serum levels of C4 (Figure 1; Tables 4-5). By contrast, no significant decrease in serum IgG and IgA was noticed (Tables 4-5).

Side effects

In patient 1, the therapeutic regimen was interrupted after the second infusion because of the development of acute left-sided amaurosis with a documented thrombosis of the retinal artery.[18]

No other acute or delayed relevant side effect or infectious complication occurred.

A symmetric panniculitis at the elbows and knees, which spontaneously resolved after one month, was noticed in patient 2 at the end of month +2.[20]

With regard to the possible negative effects of treatment on HCV chronic infection, there was no evidence of liver toxicity after treatment. By contrast, serum albumin significantly increased after treatment (Tables 4-5).

A mild increase in serum alanine or aspartate aminotransferase levels was present in 3 of 15 patients within the 3 months preceding the study and at baseline. After rituximab treatment, no further increase in aminotransferase levels was noticed in such cases, whereas normalization was noticed in one of them (data not shown). Aminotransferase levels remained within all the normal ranges in all the remaining patients with normal values at baseline.

The HCV serum viral load remained above the upper limit of detection by quantitative PCR (> 850.000 IU/mL) in 3 of 8 studied cases (patients 5, 7, and 15), increased to above such upper limit in 2 of 8 cases (170 000 and 180 000 IU/mL at baseline in patients 10 and 13, respectively), showed minimal fluctuations in 2 patients (patients 8 and 11), and decreased in one patient (patient 6, from > 850 000 IU/mL at baseline to 98 000 IU/mL at month +6).

Table 4. The course of rheumatoid factor, cryoglobulin, and immunoglobulin serum levels in the studied patients after rituximab therapy

	Median value			Decrease less than 25% or unchanged		Decrease 25% or greater		Normalization		Notes
Months	0	+3	+6	+1 to +5	+6	+1 to +5	+6	+1 to +5	+6	—
RF IU/mL	536	121	137*	1 of 14	2 of 14	9 of 14	9 of 14	4 of 14	3 of 14	Negative in patients 3, 4, 14 (+3 and +6) and 2 (+3)
Cryoglobulins mg/dL	1305	767	819*	1 of 13	4 of 13	9 of 13	8 of 13	3 of 13	1 of 13	Negative in patients 4 (+3 and +6) and 1, 14 (+3)
IgM g/L	3	1.6	1.7*	3 of 15	5 of 15	12 of 15	10 of 15	NA	NA	Decrease ≥ 25% in patients 3–9, 11, 13, 14 (+6)
IgG g/L	8.4	7.7	7.2	12 of 15	12 of 15	2 of 15	3 of 15	NA	NA	Decrease ≥ 25% in patients 3, 4 (+3 and +6) and 8 (+6)
IgA g/L	1.2	0.8	0.8	10 of 15	10 of 15	5 of 15	5 of 15	NA	NA	Decrease ≥ 25% in patients 3–6, 8 (+3 and +6)

Changes were evaluated with respect to the baseline value in any single patient.
RF indicates rheumatoid factor (positive if ≥ 20 IU/mL); and —, not applicable.
*$P \leq .05$.

Table 5. The course of C4 and albumin serum levels in the studied patients after rituximab therapy

	Median value			Increase less than 25% or unchanged		Increase 25% or greater		Normalization		Notes
Months	0	+3	+6	+1 to +5	+6	+1 to +5	+6	+1 to +5	+6	—
C4 mg/dL	2	8*	8*	6 of 14	7 of 14	3 of 14	3 of 14	5 of 14	4 of 14	Normalized at month +6 in patients 3, 4, 14, 15
Albumin g/L	3.7	3.9*	4.0*	12 of 15	14 of 15	0 of 15	0 of 15	3 of 15	1 of 15	Normalized in patients 5 (+3 and +6) and 1, 11 (+3)

— indicates not applicable; C4, complement fraction (decreased if < 10 mg/dL); albumin, low at baseline (<3.5 g/L) in 4 of 15 patients (patients 1, 5, 8, and 11).
*$P \leq .05$.

Long-term follow-up

Although the investigation of the long-term effects of rituximab therapy was not the goal of this study, follow-up after the 6-month study period is presently available in all the patients (Table 1) and is concisely reported.

Patient 1, after interruption of rituximab therapy,[18] relapsed at month +3 and died at month +12. He had been followed in another center under corticosteroid and dialytic treatment for chronic renal insufficiency. All the remaining patients are presently alive. Notably, 9 patients remained in good condition and received no further treatment for an additional 3 to 28 months after the end of the 6-month study period, whereas 5 patients were retreated with rituximab (4 weekly infusions, as at baseline in patients 3, 4, 11, 12, and 13).

Purpura disappeared and then recurred within the 6-month study period in patients 3 and 13 (Table 2), who were then retreated with rituximab and responded again. By contrast, purpura did not recur in any of the remaining 9 responders.

Ulcers disappeared and then recurred within the 6-month study period in patient 12 (Table 2), who responded again to a second course of rituximab. Ulcers recurred in one of the remaining 4 responders during additional follow-up (ie, at month +18 in patient 3) and disappeared again after repeating rituximab.

Vasculitic urticaria is still in remission in patient 2 at month +31.

In the 2 patients with long-term response in non-Hodgkin lymphoma (patients 5 and 8) response persists up to the last follow-up (Tables 1 and 2). In the third patient (patient 4, with salivary gland lymphoma recurred at month +6), autoimmune hemolytic anemia and neutropenia recurred at month +15 and responded to a second course. Recurrence of neuropathic pain and paresthesias occurred in 2 of 8 patients (patient 3 and patient 11) during long-term follow-up. Interestingly, patient 3 had been retreated with rituximab at the end of month +6 in the attempt to further ameliorate the symptomatic polyneuropathy, but additional improvement was not achieved. By contrast, a third course of rituximab given at month +18 because of recurrence of skin ulcers and of neuropathic pain was effective for both manifestations. In patient 11, both neuropathic symptoms and nephritis recurred at month +7, and both manifestations were again responsive to rituximab.

No side effects were recorded in these patients during long-term follow up or after retreatment with rituximab. However, breast cancer was diagnosed in patient 3 at month +24, and a peripheral B diffuse large cell lymphoma developed in patient 14 at month +9, in the lack of active cryoglobulinemic features.

Discussion

The present study supports the efficacy and safety of rituximab therapy in type II MC, in agreement with previous preliminary reports by our group.[18,20] Type II MC is a systemic vasculitis prevalently mediated by immune complexes, usually associated with HCV infection, and characterized by proliferation of RF-positive B-cell clones producing cryoglobulins and therefore directly playing a key pathogenetic role.[1,2] Rituximab is a chimeric monoclonal antibody that reacts with the CD20 antigen, thus directly and selectively targeting the B cells.[14-17]

There is a strong rationale for using rituximab in type II MC. Biologic evidence of effective targeting of RF-positive B-cell clones by rituximab has been reported both in preliminary clinical studies[19,20] and in the animal model.[24] In addition, the drug proved effective and safe in B-cell lymphoproliferative disorders as well as in some autoimmune diseases.[15-17]

In this study, rituximab was used at the usual 4 weekly doses[15-20] in type II MC patients unresponsive to standard treatment. In many of them, several different treatments had failed. Thus, rituximab treatment proved of major clinical impact in such patients difficult to manage. Although several treatment approaches are currently available for type II MC, ranging from antiviral drugs to corticosteroids, immunosuppressors, and plasmapheresis,[1,6] the treatment of the single patient may be quite difficult due to drug inefficacy or limited efficacy, drug contraindications, or side effects. The availability of a new therapeutic option is therefore a major advance.

Rituximab proved very effective on the cutaneous manifestations of type II MC. When present, skin ulcers healed in all the cases. The effects on purpura and urticarial vasculitis were rapid and dramatic. Although the latter manifestations do not require aggressive treatments in type II MC, their prompt disappearance after treatment underscores the efficacy of rituximab on immune complex–mediated small-vessel vasculitis. This might be relevant for the management of other systemic features associated with small-vessel vasculitis, possibly including acute or rapidly progressive organ manifestations not investigated in the present study.

Rituximab treatment may be of value in low-grade B-cell lymphoma associated with type II MC. The role of rituximab as a safe therapy to control B-cell lymphoma is well-established,[14-16] and response was noticed in patients with lymphoma and type II MC in this study. When considering the usual indolent course of type II MC–related low-grade lymphomas, the age of the patient, the concomitant vasculitis features, the other drugs in use, and the underlying viral infection, then aggressive treatments to eradicate the B-cell tumor seem much more hazardous than useful for most patients. Rituximab has the advantage of allowing avoidance of serious immunosuppression and side effects,[15,16] and it lacks direct oncogenetic properties that could favor the progression of indolent lymphomas into an aggressive lymphoma. Although a complete and long-term remission of lymphoma is obtained in a minority of cases after rituximab monotherapy,[15,16] such a response is not the main goal of treatment for many patients with type II MC when considering the whole clinical picture. Concomitant systemic vasculitic and constitutional features (eg, fever, arthralgias, and arthritis) also could benefit from rituximab therapy, as observed in this study.

Treatment of type II MC–related peripheral neuropathy and nephropathy is complex. Although the present study does not allow clear-cut conclusions for what concerns the therapeutic role of rituximab, efficacy was proved.

An improvement in subjective symptoms of sensory peripheral polyneuropathy was noticed in all our patients, despite no changes in the electromyography pattern (consistent with axonal plus myelinic damage) before and after treatment. In addition, a marked improvement in muscle strength was noticed in patients whose motor disturbances were clinically relevant at baseline. These results are consistent with (1) no further increase in peripheral nerve damage during the 6-month follow-up after treatment, (2) lack of primary demyelination as the key mechanism of nerve damage, (3) a likely improvement in axonal damage after treatment, in polyneuropathy characterized by a primary axonal damage with secondary myelinic damage (as documented in type II MC–associated polyneuropathy).[25,26] Improvement in axonal damage is suggested by reduced neuropathic symptoms but, when present, it cannot be directly demonstrated by electromyography,[23] whereas recovery in secondary myelinic damage (after improvement in axonal damage) occurs slowly and may be detected by electromyography after a longer follow-up.[23] Controlled studies versus placebo with a long-term follow-up are required to better address this issue in type II MC. A placebo effect is unlikely in our patients, given that amelioration in symptoms lasted several months.

A second issue is represented by the duration of polyneuropathy, which was of several years in this study. Minor response may be due to irreversible neural damage in this subset of patients. Thus, future studies are worthwhile to investigate the effects of rituximab in early-onset type II MC–associated neuropathies, which may better respond to treatment.[12] The role of additional mechanisms of nerve damage in type II MC finally should be considered,[25,26] and the targeting of immune complex–mediated damage might not represent the only approach for the most effective treatment.

With regard to glomerulonephritis, persistent disappearance of proteinuria was observed in one of our 2 treated cases, where nephritis was of recent onset. In another patient no effect was noticed, but treatment schedule with rituximab was interrupted after the first 2 infusions,[18] and nephritis was of long duration. Additional studies are then required, and early-onset nephritis should be included. This is a relevant issue when considering that nephropathy is one of the major causes of death in type II MC.[11]

Finally, in this study rituximab proved effective in reducing or avoiding corticosteroids in all the type II MC patients who required chronic steroid therapy. Such a steroid-sparing effect is relevant for any new drug proposed for any chronic inflammatory disease that is steroid dependent, with additional benefits for patients with concomitant viral infection such as type II MC.[4] A possible synergistic role for steroids and rituximab has been recently suggested[27] and may have contributed to the positive results in some of our cases. Response to rituximab was however observed also in the lack of steroids. This issue should be further investigated, considering that steroid sparing remains a cornerstone in the long-term management of MC.

For what concerns the key laboratory features of type II MC, this study provides evidence that rituximab effectively targets the RF-positive proliferating B-cell clones sustaining the immune complex–mediated tissue damage, thus supporting the clinical efficacy observed. A significant decrease in mean serum RF levels and cryoglobulins was observed, with negativization in some cases, accompanied by an increase in serum C4 levels in about half the cases. Because serum IgM levels were significantly decreased after rituximab treatment, whereas IgG and IgA were not, the possibility of a selective inhibition of IgM-positive $CD20^+$ plasma cells producing autoantibodies, as recently proposed by Treon and Anderson, may be hypothesized.[28]

Interestingly, although B-cell depletion was rapidly achieved in the peripheral blood and persisted up to month +6 in all the studied patients, bone marrow B-cells were within the normal range (though reduced with respect to baseline) in 5 of 7 studied cases at month +6. This is consistent with the evidence of persisting positive (though reduced) serum RF and cryoglobulins at month +6 in most patients, that is, with the notion that rituximab is effective in type II MC but does not allow a complete suppression of the B-cell–mediated autoimmune response.

The present results also should be considered in the light of the currently available therapeutic approaches to inhibit cryoglobulin synthesis and pathogenetic effects.[1,6,12] Molecular evidence of antigen-driven B-cell proliferation is definitely provided in HCV-associated type II MC and B-cell lymphoma, and HCV appears as the key trigger.[29-31] Of note, antiviral treatment against HCV proved effective in type II MC.[1,3-8] Reduction of RF, cryoglobulins, and lymphoproliferation has been reported, in particular, in the subset of patients where HCV RNA became negative after treatment.[3-8] On the other hand, antiviral therapy may also fail, or may prove poorly tolerated or counterindicated, in many patients with type II MC.[1-12] Furthermore, RF-positive B-cell clones may be stimulated by immune complexes with IgG bound to quite different antigens,[32] and clinical studies demonstrate the persistence of serum RF and cryoglobulins in most patients with type II MC despite the HCV RNA negativization.[3-8] Thus, present data do not support the concept that antiviral therapy alone may completely suppress the RF-positive B-cell response in type II MC, at least in the short term, and additional therapeutic options are required.[33]

On the other hand, current treatments directed to the B-cell arm of autoimmunity in type II MC, that is, high-dose steroids, cyclophosphamide, or plasmapheresis, are difficult to manage and imply a high risk of serious side effects in long-term use.[12,13] They may also prove ineffective or contraindicated as induction therapy. For this reason, present data on the efficacy and safety of rituximab in type II MC, although preliminary and to be verified in additional trials, are of major interest. Overall, rituximab appeared well-tolerated and safe in patients with type II MC. Few and minor side effects were noticed in our cases, in agreement with previous results in a large number of patients with B-cell lymphoma and in patients with autoimmune disease.[14-20] Serious infections, which represent a major cause of death in type II MC patients under standard immunosuppressive treatment, were never observed. In addition, no worsening of liver disease was noticed, thus supporting the safety of rituximab with regard to the underlying HCV infection in type II MC.

Of note, one of our patients developed a thrombosis of the retinal artery after the second infusion of rituximab.[18] Rituximab administration has not been associated with arterial or venous thrombosis in large series of patients, however. Careful investigation of clinical and laboratory risk factors for arterial thrombosis should be performed in any patient with type II MC, and aspirin prophylaxis given, if possible, when these are present. Follow-up should be stricter in patients treated with rituximab, in the lack of follow-up data in a larger number of patients.

In conclusion, the present study indicates that rituximab may represent a safe and effective therapeutic option for type II MC. Future controlled, randomized studies are needed to define drug

indications and the cost-efficacy profile in the different systemic features of the disease, if compared to standard treatment. The duration of response to rituximab also should be carefully assessed, but follow-up data from this study are encouraging. The simultaneous blockade of both the infectious trigger HCV and the activated RF-positive B-cells by means of combined therapy (antiviral plus rituximab) is also a challenge to better suppress the disorder with fewer side effects.

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